DIOXINS IN FOOD: AN AGRICULTURAL PERSPECTIVE

Janice K. Huwe
USDA, ARS, Biosciences Research Laboratory, P.O. Box 5674 University Station, Fargo, ND, 58105

The Dioxin Problem

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants which persist and bioaccumulate through the food chain. They are often collectively referred to as "dioxins" because of their similar structures, chemical properties, and mechanism of action in biological systems. PCDDs, PCDFs, and the non-ortho and mono-ortho substituted PCBs are planar, highly lipophilic compounds (Figure 1). Although 210 different PCDDs and PCDFs are possible with one to eight chlorines, only seventeen of these congeners are considered toxic. Toxicity and persistence are determined by structure, with lateral substitutions (positions 2,3,7, and 8) imparting the highest degree of toxicity. Of the 209 possible PCBs only twelve have any dioxin-like toxicity. These are all non-ortho and mono-ortho substituted compounds. In order to define the relative potency of dioxin-like compounds, toxic equivalency factors (TEFs) based on in vivo and in vitro studies have been defined (Safe 1990). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic dioxin and has been assigned a TEF value of one; TEFs for other dioxin-like compounds are based on activity relative to TCDD and have been updated as more experimental data are collected (NATO/CCMS 1988; Ahlborg et al. 1994; Van den Berg et al. 1998). Table 1 summarizes the TEF values most commonly used for human risk assessment: the 1988 NATO/CCMS set also called I-TEFs, the 1994 WHO set for PCBs (Ahlborg et al. 1994), and the 1998 WHO set (Van den Berg et al. 1998). The major change in the current 1998 TEFs is the increased weighting of PeCDD from 0.5 to 1.0. Using the TEF concept, toxicity

equivalents (TEQs) can be calculated for any sample by summing the TEF-weighed concentrations of each dioxin-like compound.

Underlying the toxic equivalency theory is the assumption of a common mechanism of action for dioxin-like compounds in which toxic responses occur as a result of initial binding to the aryl hydrocarbon (Ah) receptor (Hankinson 1995; Birnbaum 1994). The cascade of events which follows is thought to produce multiple health effects such as carcinogenicity, endocrine disruption, developmental and reproductive problems, immunotoxicity, neurological alterations, chloracne, and, at high enough doses, wasting syndrome and death (Schecter, Ed. 1994). The doses at which effects can be observed depends both on species and endpoint. Lethal doses of TCDD range from ug/kg for guinea pigs, LD_{50} = 0.6 ug/kg (Schwetz et al. 1973) to mg/kg for hamsters, LD_{50} = 1160 ug/kg (Olson et al. 1980). Induction of cytochrome P-450 1A1 (CYP-1A1) mRNA is a particularly sensitive endpoint and is measurable after a single dose of 100 pg TCDD/kg in rats (Sewall et al. 1995). Immunological and developmental effects have been seen in laboratory animals at single or multiple doses below 1 ug/kg body weight (Kerkvliet 1995; Peterson et al. 1993). TCDD has also been shown to cause cancers in animals at chronic exposures as low as 1 ng/kg body weight/day (Huff et al. 1994).

In humans, dioxin's effects have been evaluated in occupationally or accidentally exposed cohorts. Pregnant women exposed to PCDFs by contaminated oil in Yu-Cheng, Taiwan, showed elevated CYP-1A1 enzyme levels compared to controls. The level of induction was similar to that cause by TCDD in rats (Lucier 1990). Children of these women exhibited developmental effects similar to the effects seen in mice and monkeys exposed to TCDD (Peterson et al. 1993). A number of epidemiology studies have shown overall increases in

cancer mortality due to TCDD exposure (Huff et al. 1994), and recently the International Agency for Research on Cancer has named TCDD a known human carcinogen (IARC 1997).

In light of continuing concerns about dioxin-related health risks, the U.S. EPA has been reassessing the impact that dioxins and related compounds have on society. The process has been ongoing since 1991 and has involved scientists from government, academia, industry, and public interest groups. The review of new data and research findings has been extensive and has delayed the finalization of the document. A recent draft of the reassessment was released for comment this past summer. Thus far no regulatory actions have been taken based on the reassessment (Birnbaum and Farland 1996).

As part of the review on dioxins, EPA and other groups have cataloged the major sources of dioxins. Major sources are combustion and incineration processes along with smelting operations, and the pulp industry. In the early 1990's medical and municipal waste incinerators had estimated PCDD/PCDF emissions of 0.7 - 5 kg TEQ/yr and 2 - 3 kg TEQ/yr, respectively (Thomas and Spiro 1996). EPA estimates that annual emissions have decreased from 13.5 kg TEQ/yr to 2.8 kg TEQ/yr between 1987 and 1995 mainly due to improvements of incinerator performance and removal of highly polluting incinerators (U.S. EPA 2000). Other regulations including bans or restriction on the production and use of chemicals such as pentachlorophenol (PCP) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), the phase out of lead in gasoline, and the elimination of chlorine bleaching in the pulp industry have also contributed to reducing levels of PCDD/PCDFs.

In addition to the major sources, there are many diffuse sources that may be difficult, if not impossible, to regulate (i.e. home heating, diesel engines, forest and grass fires, backyard

burning). Backyard burning of household waste was recently shown to produce more PCDD/PCDFs per mass burned than a typical modern municipal waste incinerator (Lemieux et al. 2000) and was estimated to account for 22% of dioxin emissions in North America from 1996-1997 (NACEC 2000). Unidentified sources also remain as indicated by budget calculations on sources and deposition which often show that depositions exceed known emissions (Rappe and Kjeller 1994; Brzuzy and Hites 1996). Baker and Hites (2000a) have proposed that reactions of atmospheric pentachlorophenol (PCP) contribute to the overall levels of dioxins. Another contribution may come from naturally formed PCDD/PCDFs which have recently been suggested by findings in archived deep soils (Green et al. 2000) and clays from the southern U.S. and Germany (Ferrario et al. 2000a).

Unlike PCDD/PCDFs, PCBs were intentionally produced as industrial fluids and plasticizers. Hundreds of thousands of metric tons were produced in the U.S. until 1977 when production was banned. As a result of their chemical stability and the reservoirs that still exist, PCBs are ubiquitous in the environment and, like PCDD/PCDFs, bioaccumulate.

Following the trend of reducing PCDD/PCDF/PCB sources, the levels in the environment have decreased as well. Sediment cores from Lake Siskiwit (a lake on Isle Royale in Lake Superior) were analyzed for PCDD/PCDFs for the time period 1888 - 1998 (Baker and Hites 2000b). PCDD/PCDF levels peaked in the early to mid-80's and declined 50% by 1998. Herring gull and guillemot eggs from the Great Lakes and Baltic regions, respectively, have shown similar decreases in PCDD/PCDF and PCB levels (Hebert et al. 1994; de Wit et al. 1994).

Because of the decline in environmental levels, background human exposure to dioxin-like compounds has also declined. Humans are exposed to dioxins mainly through their diet

with animal products accounting for almost 90% of this exposure (Fürst 1993; Fries 1995). Estimates of dietary exposure in Europe have shown that daily I-TEQ intakes decreased by 60% from the 1980's to the mid 1990's (Harrison et al. 1998; Malisch 1998). The current European daily intakes were estimated to be 1 - 3 pg I-TEQ/kg body weight, which is comparable to the range of 0.3 - 3.0 pg I-TEQ/kg body weight estimated in a study of the U.S. population (Schecter et al. 1994). The decline in body burdens are also indicated by human blood and milk monitoring. In Germany the average blood level of PCDD/PCDFs decreased 50% to 20.7 pg I-TEQ/g lipid from 1989 - 1998 (Wittsiepi et al. 2000); human milk levels decreased 30% to 20.7 pg I-TEQ/g lipid from 1987 - 1992 (Fürst 1993). Swedish human milk showed a 70% decline in PCDD/PCDFs and PCBs from 1972 - 1997 reaching 26.4 pg I-TEQ/g lipid; PCBs accounted for 52% of the TEQ (Norén and Meironyté 2000). Body burdens in the U.S. have not shown a consistent trend. Perhaps due to the small numbers of samples analyzed, blood levels of PCDD/PCDFs appeared to remain steady at ca. 27 pg I-TEQ/g lipid from the 1980's up to 1996, while milk showed a decline from 20 to 8.2 pg I-TEQ/g lipid during this time (Schecter et al. 1996).

Due to the risks associated with dioxin exposure, the World Health Organization (WHO) has recommended a total daily intake of 1 - 4 pg I-TEQ/kg body weight (van Leeuwen et al. 2000). The U.S. EPA has set a one/million cancer risk level at 0.006 pg I-TEQ/kg body weight/d (Birnbaum and Farland 1996). Based on the current estimates of daily intake in the U.S., the general population falls within the recommended guideline of WHO but is well above the virtually safe dose set by EPA. Because dioxins can show measurable biological effects at extremely low levels (ppt or ppq), there is concern at EPA that our current intakes and resulting

body burdens may produce subtle adverse effects in the population and especially in subsets of the population who may be most sensitive to dioxins' effects.

Levels in Foods

Given that animal products are the primary source of human intake and exposure, several models have been developed to predict the accumulation of dioxins in livestock (Fries and Paustenbach 1990; Lorber et al. 1994; McLachlan 1997). In all cases air deposition of dioxins onto forage and, to a lesser extent, soils is assumed to be the major route of exposure for livestock. Although the models predicted beef and milk dioxin levels in fairly good agreement with observed values, several deficiencies were noted. Congener specific data for rural air levels are minimal, as are data on the actual levels in beef and other livestock. Only one study was found which provided bioavailability and bioconcentration factors for multiple PCDD/PCDFs, and that study had been conducted with one lactating cow (McLachlan et al. 1990). No studies have been done to evaluate specific practices such as feedlot fattening which may affect dioxin levels in beef before slaughter. More research in all of these areas are needed to validate the models and strengthen their predictive capabilities. It should also be noted that these models predict background dioxin exposure from air deposition, not contaminations which may arise from other sources.

Dioxin levels have been surveyed in foods in the U.S. on only a limited basis because of the costs associated with the analysis. Statistically-designed surveys of beef, swine, and poultry were conducted by USDA and EPA in the mid-1990's and involved 60-80 samples from randomly selected slaughter houses (Winters et al. 1996; Lorber et al. 1997a; Ferrario et al. 1997). Using the 1998 TEFs and non-detects set equal to half the limits of detection, beef back

fat had an average PCDD/PCDF level of 1.08 ppt TEQ; pork belly fat averaged 1.48 ppt TEQ; and poultry abdominal fat averaged 0.83 ppt TEQ. The dioxin-like PCBs contributed another 0.47, 0.06, and 0.36 ppt TEQ to beef, pork, and poultry, respectively. In each survey adipose tissue was sampled and assumed to give a good measure of the levels which would be found in retail meats because dioxins are thought to be distributed equally into lipid compartments. A few studies have been done to investigate this assumption. Ferrario and Bryne (2000b) analyzed various chicken samples and compared dioxin levels in breast and thigh meat to adipose tissue on a lipid adjusted basis. All matrices were equivalent. In cattle, however, intramuscular lipids (i.e. ribeye) may contain more TCDD, more of the higher chlorinated dioxins and furans, and more of certain PCBs than the back fat or perirenal fat (Lorber et al. 1997b; Feil et al. 2000). The use of back fat as a sampling matrix would then lead to underestimates of the dioxin levels in edible meats.

In a geographical survey designed by the USDA-ARS, over 160 beef samples from 13 states across the U.S. were analyzed for PCDD/PCDFs to determine regional variations in the background levels of dioxins. In order to obtain samples from cattle that were raised and fed in a given location and to increase compliance with the experimental sampling protocols, samples were collected from State and Federal experiment stations. Although data from this survey has not been fully evaluated, several locations produced animals with noticeably high levels of dioxins, 7.8 - 52.9 pg I-TEQ/g lipid (Feil et al. 1997). Investigations of these sites found that the animal feed had non-detectable levels of dioxins but the posts and feed bunks had significant levels of some PCDD/PCDFs. The pattern of dioxins found in the wood indicated that pentachlorophenol (PCP) may have been used as a wood preservative; PCP was later identified

in these samples (Fries et al. 1998). Although the use of PCP was restricted in the 1980's, PCP was heavily used on farms as a wood preservative in the late 1970's (Schull et al. 1981). Because PCP is a very effective preservative, treated wood can last for decades with most of the original PCP remaining in the wood, even after 25 years (www.awpi.org/pentacouncil). The extent of buildings or fences which may contain PCP-treated wood today is, to our knowledge, not known. Therefore, the impact of PCP-treated wood on dioxin levels in meat and dairy products can not be adequately evaluated at this time.

Reported dioxin levels in U.S. dairy and farm-raised fish have been minimal. A recent EPA survey of milk in the U.S. showed an average of 0.82 pg I-TEQ/g lipid due to PCDD/PCDFs and another 0.50 pg I-TEQ/g lipid due to PCBs (Lorber et al. 1998). Geographically the southwestern U.S. appeared to have the lowest dioxin levels and the southeast the highest (ave. 0.51 and 1.13 pg I-TEQ/g lipid, respectively). Reported dioxin levels in farm-raised catfish in the U.S. have all stemmed from a contaminated feed incident. Lipid weight concentrations in these catfish were 6.5 to 44.9 pg I-TEQ/g with PCDD/Fs accounting for 90% of the TEQ (Fiedler et al. 1998). Catfish raised on diets which did not contain the contaminated ball clay had TCDD levels about one fifth those of the contaminated fish (Hayward et al. 1999). As a comparison, wild fish in the U.S. have been monitored for dioxins by FDA and EPA for the past 15 years (Firestone et al. 1996; Kuehl et al. 1994). Fish collected in the late 1980's had an average TCDD level of 6.9 ppt wet weight, while by the mid 1990's TCDD was not detected in most fish (LOD = 1-2 ppt).

In addition to analyzing samples from production sites, several market basket surveys have analyzed goods purchased at retail stores for PCDD/PCDF levels. These results are

tabulated in Tables 2 and 3 on a lipid weight basis and a whole weight basis and compared to values reported in several European countries. Considering the different methods used to report non-detect values, data between the different studies in Europe and the U.S. are comparable. The levels found by Schecter et al. (1997) in samples from across the U.S. appear to be somewhat higher than those found in Mississippi (Fiedler et al. 1997) on a lipid weight basis and may reflect regional variations in dioxin levels. Comparison of these two data sets shows the importance of reporting levels on a wet weight rather than lipid weight basis when calculating actual consumption amounts. A food item with relatively high dioxin concentrations on a lipid basis may have a much lower value when converted to a wet weight or serving portion size if the fat content is low. In the Schecter study meat and cheese samples all had lower fat content than those sampled by Fiedler resulting in similar concentrations of dioxins on a whole weight basis but not on a lipid weight basis. Tables 2 and 3 also point out the impact of switching to the newest set of TEFs. For food samples almost all TEQs increased due to the increased contribution of 1,2,3,7,8-PeCDD (TEF changed from 0.5 to 1.0). In the two surveys where PCBs were measured, the PCB TEQ averaged 50% of the dioxin TEQ in the U.S. (Schecter et al. 1997) and was equivalent to the dioxin TEQ in the Netherlands with the exception of Dutch fish which had almost 300% more PCB TEQ than dioxin TEQ (Theelen et al. 1993).

While many of the surveys have shown low background levels of dioxins, occasionally a highly contaminated sample was found. In the ARS geographical survey, high levels of dioxins were traced back to PCP-treated wood used at the rearing facilities. In the EPA survey on poultry, two chicken samples were found with levels well above the average (22 and 26 ppt). The origin of this contamination was ball clay which had been added as an anticaking agent to

soy meal in the feed (Ferrario and Bryne 2000b). This same contaminated feed was also used by the catfish industry and resulted in dioxin levels of ca. 40 ppt in catfish from Arkansas (Fiedler et al. 1998). The unique congener pattern identified in the ball clay was not similar to any known anthropogenic source and led to speculation that the dioxins in the clay were naturally formed (Ferrario, et al. 1999; Rappe et al. 1998). .

Several dioxin contaminations have occurred recently in Europe. In 1998 during routine monitoring, dairy products were identified which had dioxin levels that were 2 to 4 times higher than normal. The source of the contamination was traced to citrus pulp used as a cattle feed component (Malisch 2000a). The citrus pulp and contaminated feeds were immediately removed from the market. In another incident, PCB/PCDD/PCDF-contaminated oil was added to recycled fat used as an additive in animal feeds. The tainted feeds contaminated Belgian poultry, dairy, and meat and were discovered only after toxic effects were seen in chickens. Animals and products were quarantined, recalled, and eventually destroyed. The incident led to international recalls and bans against Belgian products. All of these contamination episodes point out not only the importance of regular monitoring of the food supply for dioxins, but also our lack of understanding of all dioxin sources. Ball clay and citrus pulp were not obvious matrices in which to find dioxins; one being a predominantly inorganic material and the other of plant origin.

The unique congener patterns (fingerprints) found in some of the contamination incidents helped point to the sources responsible. For example, PCP-treated wood and ball clay have characteristic fingerprints which carried over, to a great extent, to the animal tissues. Distinctive fingerprints have been cataloged for most major dioxin sources (Cleverly et al. 1997); however,

data on the biotransformations of these patterns in the animal system are lacking. Only two studies have reported bioavailability and bioconcentration factors for PCDD/PCDFs in beef (Feil et al. 2000) and chickens (Stephens et al. 1995). A better understanding of these transformation factors may facilitate source attribution.

Methods to Reduce Exposure

Once animals have been contaminated with dioxins, no practical methods are available to reduce the body burdens. Therefore, in most of the contamination incidents, animal products have been removed from the market and destroyed. One common strategy to reduce levels of undesirable compounds in exposed animals is by depuration. For example, drugs or therapeutics used in animal care are withheld for a given period of time before animals or products are ready for market in order to assure that the compound has been cleared from the animal's system and provides no exposure risk to consumers. In the case of dioxins, however, long half-lives in the animal make withdrawal periods impractical. Estimates of the half-lives of PCDD/Fs in milk range from 40 to 190 days (Jensen and Hummel 1982; Firestone et al. 1979; Tuinstra et al. 1992). In beef cattle adipose tissues these half-lives are even longer at 100-200 days (Startin et al. 1994; Thorpe et al. 1999). Based on a limited amount of data, dioxin half-lives in chickens appear to be 25-60 days in adipose and eggs (Stephens et al. 1995).

Several methods have recently been reported which may increase clearance of dioxins from animal systems. The use of a leanness enhancing agent, clenbuterol, was investigated in rats as a means to reduce dioxin body burdens after an acute exposure. Compared to controls, rats fed clenbuterol-supplemented feed for 10 days after a dioxin exposure had 30% less fat and

30% less total dioxin burden (Shappell et al. 2000). In another study the addition of an insoluble evacuation substance (chlorophyllin-chitosan) to the feed was studied in mice as a means of promoting dioxin excretion (Aozasa et al. 2000). A diet containing 1% chlorophyllin-chitosan was fed to mice after a single oral dose of HxCDD. Excretion of HxCDD increased 350% and deposition into adipose tissue decreased 30% compared to controls. Approaches such as these may eventually prove to be practical and economical if the necessary supplements are inexpensive, cause no adverse growth or health effects, and are easily incorporated into animal husbandry routines. All of these strategies are still in the research stage and a long way from implementation.

At present the best way to reduce dioxin levels in livestock is to minimize exposure. Known sources of dioxin such as PCP-treated wood should be identified and removed from areas where animals may come into contact with them. Feed and feed components which have been identified as contaminated should be removed from all markets. A few studies have shown that feed ingredients from plant origin are generally lower in dioxins than those of animal origin. Rappe et al. (1998) found an average of 142 pg TEQ/kg dry weight for plant feed materials (excluding soymeal contaminated by ball clay) but 615 pg TEQ/kg dry weight for animal meals. Animal and fish lipids used in feeds had even higher levels, 1040 and 2750 pg TEQ/kg, respectively (Malisch and Fürst 2000b; Eljarrat et al. 2000). The substitution of plant meals, e.g. soy, for animal or fish meals may prove to be an effective means to lowering dioxin intake in livestock and aquaculture; however, further research is needed to confirm and optimize this strategy.

Other agricultural practices which have not been evaluated with respect to their impact

on dioxin levels in livestock include the application of sewer sludges to pasture lands, the burning of pesticide-laden crop lands, forest and range fires, and differences between grazing and pen-fed animals. One study in Germany showed the potential for increased PCDD/PCDF/PCB levels in milk from dairy cattle raised on a farm which applied sludge to the fields (McLachlan et al. 1994).

The agricultural industries have already made progress towards reducing dioxin levels by the production of leaner beef and pork. Because dioxins accumulate in fat stores, the production of leaner meats should decrease the overall dioxin body burdens in these animals. As a consumer, maintaining a low fat diet and trimming excess fats are ways to further diminish dioxin intake. Following the USDA recommended dietary guidelines should provide one means to lower dioxin exposure: make grains and rice your primary base, eat five servings of fruits and vegetables a day, limit calories from fat to 30% of the total and calories from saturated fats to 10% or less.

Another method for consumers to reduce dioxin intake is through cooking practices. Several studies on the effects of cooking on dioxin levels in foods have been published. In all cases the amounts of dioxins in a serving portion were decreased by 30 to 70% on average by various cooking methods. Stachiw et al. (1988) used restructured carp fillets containing 50 to 100 ppt of 2,3,7,8-TCDD to evaluate roasting and charbroiling. The largest reductions in TCDD (59 - 70%) were found for well-done fillets. Zabik and Zabik (1995) found baking, pan frying, and deep fat frying of fish fillets similar to broiling in the amounts of TCDD removed. Removal of the skin from fillets also increased TCDD losses during cooking. The amounts of TCDD present in the original fillets had little effect of the percentage loss in these studies (TCDD range

0.5 to 100 ppt).

Other dioxin, furan, and PCB congeners have been shown to decrease by a percentage similar to TCDD in broiled hamburger, bacon, and catfish (Schecter et al. 1998) or in pan-fried hamburger (Petroske et al. 1998). In hamburger, levels of each congener decreased 30 to 50% while the total TEQ decreased 48%. Decreases correlated well with the loss of lipids during cooking which averaged 42 and 48%, respectively in each study. Bacon and catfish lost an even higher percentage of congeners due to the higher percent of lipids cooked out: TEQ down 56% in bacon with a concomitant loss of 75% lipids; TEQ down 58% in cooked catfish with 62% of the lipids removed. In the mass balance study by Petroske at al. (1998), the fats and juices cooked out of the hamburger contained the balance of dioxins and furans. Total recoveries were 82 to 99% for all congeners, indicating no formation from the frying process and little thermal degradation of any congeners.

From these studies, cooking appears to be a reliable way to remove dioxins from meats provided the fats and juices are discarded. The removal of skin from fish fillets is another means of enhancing the loss of dioxins through cooking. No studies have yet been reported which assess the effects of the use of contaminated oil or grease to fry meats or other food products. Only one mass balance study has been reported which used pan-fried hamburger; other such studies on different cooking methods may be warranted to definitively rule out formation of dioxins or furans by cooking processes.

Monitoring

In Europe recommended levels have been established for dioxins in foods and feedstuffs. Germany and the Netherlands have limits on the levels of dioxins acceptable in dairy products: 5

and 6 pg I-TEQ/g lipid, respectively (Liem and Theelen 1997). Tolerance levels of 500 pg/kg have recently been set for certain feed additives in Europe (Eljarrat et al. 2000). In the U.S. a temporary action level of 1 ppt TCDD whole weight was set by FDA during a 1997 chicken contamination crisis.

The only way to ensure that foods are low in dioxins and remain low is through routine monitoring. Monitoring programs provide an estimate of the background levels found in different food groups. Once a baseline has been established, following trends in dioxin levels can indicate problems or progress. Decreasing dioxin levels would indicate the effectiveness of regulations on point sources and of improved agricultural practices. Increasing dioxin levels would alert agencies to a contamination or to an unidentified source, and remediation steps could be quickly taken. Because livestock are mainly exposed to dioxins through their diet, feeds and feed ingredients are practical monitoring points, especially for industries such as poultry and aquaculture where feed sources are controlled. Dioxin analyses of feed ingredients are often less expensive than for food products because the lower lipid content of feed ingredients may require less rigorous cleanup methods. For grazing animals, feed sources are not as easily controlled. Therefore, for beef and dairy, meat and milk are the most logical samples for monitoring. EPA, FDA, and FSIS continue to build databases on dioxin levels in foods and animal feeds; however, the expense and time required for dioxin analyses limit the number of samples which can be assayed.

In order to make routine screening for dioxins feasible, faster, less expensive methods of analysis must be found. The current cost of a PCDD/F analysis is $800-1200 per sample. Because dioxins are present at extremely low levels i.e. part per trillion (ppt) or even part per

quadrillion (ppq), samples require extensive cleanup before detection; this contributes to the cost and also the time required for the analysis. An established cleanup method for adipose tissue includes a sulfuric acid treatment to digest the fats followed by multiple chromatography steps using acid, basic, and neutral silica gel, basic alumina, and carbon columns. In our laboratory this procedure takes 1 ½ days and consumes 2.2 liters of organic solvents and about 40 mL of sulfuric acid per sample. The other cost factor in dioxin analyses involves detection of multiple PCDD/PCDF congeners at the ppt level for which isotope dilution techniques utilizing high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) are currently the only acceptable methods (U.S. EPA Methods 1613 and 8290). Mass spectrometers capable of meeting the HRGC-HRMS performance criteria are expensive (ca. $500,000) and require an experienced, full-time operator to maintain and operate the system. The need for isotopically labeled standards also adds to the analysis cost.

Improvements which provide either faster, more efficient cleanup procedures or rapid, inexpensive screening assays will help to lower the costs of the analysis. Although the basic sample cleanup has not changed much since it was first reported by Smith et al. (1984), automation has decreased the sample preparation time. The Center for Disease Control and Prevention (CDC) along with Fluid Management Systems (FMS) have automated the procedure to reduce personnel time and solvent usage (Lapeza et al. 1986; Turner et al. 1994, 1998). Recently FMS has introduced high capacity silica cartridges which replace the manual processing steps needed to remove lipids from samples (Focant et al. 2000). With the most recent improvements, organic solvent consumption diminishes 0.6 liters per samples. In addition, no handling of concentrated sulfuric acid will be required, and the number of samples

processed in one day should double.

Immunoaffinity chromatography (IAC) is another approach which has been investigated to simplify dioxin cleanup. Immunoaffinity columns have been generated from anti-dioxin antibodies and shown to selectively bind dioxins from serum and milk samples (Shelver et al. 1998, 1999). Although milk samples required a delipidation step prior to IAC, serum was directly applied to the immunoaffinity columns. A monoclonal antibody column showed acceptable recoveries and reliable quantitation for five of the most toxic dioxins and furans in a serum matrix (Huwe et al. 1999; Shelver et al. 2000). These five dioxins and furans represented 70% of the total TEQ in the samples. The procedure required less than 2 hours for the entire cleanup, used <10 ml of organic solvent, and showed promise as a high throughput, environmentally-friendly, inexpensive method of dioxin cleanup. The application of IAC to PCB analysis has shown similar potential in a preliminary study (Concejero et al. 2000). Limitations of IAC include its incompatibility with high fat matrices and the lack of selectivity for all 17 toxic congeners. These problems may be overcome by using delipidation steps prior to chromatography and by incorporating new antibodies into the column which have specificity towards other PCDD/PCDF/PCB congeners.

The development of screening assays for dioxins to complement expensive HRGC-HRMS is another way to reduce monitoring costs. Inexpensive initial screens could be used to analyze a large number of samples; only those samples which had levels of dioxins above a threshold value would be more rigorously analyzed by HRGC-HRMS to determine congeners patterns and exact TEQ. Two types of screening techniques being explored are *in vitro* Ah receptor-based assays and immunoassays. A typical receptor-based system is the chemically-

activated luciferase expression (CALUX™) bioassay which utilizes a recombinant cell line (Garrison et al. 1996). The CALUX™ assay produces a luminescent response when compounds actively bind to the Ah-receptor which can be measured with a commonly-available spectrometer. Because dioxin-like compounds bind to the Ah-receptor as the first step in toxicity, compounds which produce a response in the CALUX™ bioassay are considered dioxin-like. The assay's response is interpreted as a CALUX TEQ by correlation to a TCDD standard response curve. The CALUX™ assay has been used to measure dioxin-like compounds in plasma, milk, animal fats, feeds, soils, and ash (Murk et al. 1997; Bovee et al. 1998; Hoogenboom et al. 2000; Brown et al. 2000). The current limit of detection is ca. 50 fg TCDD; in actual biological matrices the LOD has been reported at 0.5 ppt with some cleanup needed to remove lipids before analysis (Van Overmeire et al. 2000). When compared to HRGC-HRMS-calculated TEQs, CALUX TEQs correlated well on a logarithmic basis ($r = 0.82$ to 0.97), generally overestimated the actual TEQs, and gave 1 to 4% false negatives.

Another approach to screening is with immunoassays. Polyclonal and monoclonal anti-dioxin antibodies have been used to develop radioimmunoassays (Albro, et al. 1979) and enzyme immunoassays such as ELISAs (Vanderlaan et al. 1988; Harrison and Carlson 1997; Sanborn et al. 1998;). Although detection limits for these assays were in the 10 to 25 pg TEQ range with standards, with actual sample matrices the limits were over 100 ppt TEQ even after extensive sample cleanup. Recent improvements have demonstrated the potential for detection down to the 5 ppt level in fat and milk matrices by immunoassays (Harrison and Carlson 2000; Sugawara et al. 2000). In these studies, correlation of the ELISA-calculated TEQs to the HRGC-HRMS-calculated TEQs was good ($r > 0.9$); however, TEQs were underestimated by one half or more in

most cases.

Screening assays have made rapid progress in recent years, but still present a number of challenges for dioxin analysis. Because these assays are aqueous-based systems, lipid removal and solubility are critical to allowing interactions between the binding sites and dioxins. Matrix interferences will differ from sample to sample. No internal standards are employed to determine recoveries from cleanup procedures which can lead to underestimated TEQ values. Other complications arise due to the nature of the binding events. Receptor-based assays detect all compounds which activate the receptor not just the dioxin-like ones, which tends to overestimate TEQs. The antibodies currently used for ELISAs are limited in the number of congeners that they recognize, tending to underestimate TEQs. In spite of these problems, recent results have demonstrated that these assays can function as inexpensive screening tools if care is taken to control standards and matrix interferences.

Conclusions

Levels of dioxins in the environment and food supply have been declining since the 1980's, so that the current average daily intake for Europeans and Americans appears to be within the WHO recommended values of 1-4 pg I-TEQ/kg body weight. In the U.S., surveys of samples from slaughtering plants and grocery stores have shown generally low background levels of dioxins (<3 ppt lipid weight) in food products. USDA, together with EPA and FDA, need to continue to collect such survey data on raw commodities, feeds, processed and prepared goods from the marketplace, and fully cooked items to determine the impact of typical practices on dioxin levels in a farm-to-table continuum. These data will establish the baseline levels of

dioxins in U.S. foods and identify practices which may add to those levels. As screening assays become more reliable and costs of analyses decrease, routine monitoring of dioxins will become more feasible and result in an even safer food supply. Samples which show elevated dioxins will quickly be identified, removed from the market, if necessary, and investigated in trace-back studies to discover the source of contamination.

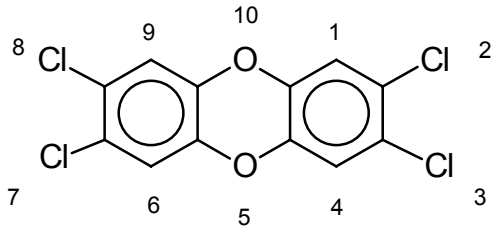
As the major sources of dioxins are regulated and controlled (i.e. incinerators), other minor sources begin to predominate in their contribution to total environmental dioxins. Several agricultural practices which need to be evaluated as potential contributors to the dioxin pool include the use of sewage sludge for fertilizing fields, agricultural burning practices, and animal husbandry issues. Included in the latter category are selections of certain feed components (e.g.. animal and fish byproducts), grazing styles which may result in larger intakes of contaminated soils by livestock, and the use of PCP-treated wood in barn facilities. One way to obtain information on the extent of PCP usage on farms may be through the National Animal Health Monitoring System (NAHMS) questionnaires which are periodically sent to farmers by APHIS (personal communication with Judy Akkina). If PCP-treated wood was found to be quite prevalent, educational materials or programs emphasizing the potential risk of dioxin contamination from PCP could be distributed or initiated. Investigations should also continue into the possible *in vivo* and *in vitro* formation of dioxins from precursors such as PCP or predioxins, the occurence of dioxins from natural sources, and currently unrecognized sources of dioxins.

Basic research into the biotransformations of dioxins in animal systems is an area which needs more attention. Knowledge of absorption, disposition, metabolism, and excretion

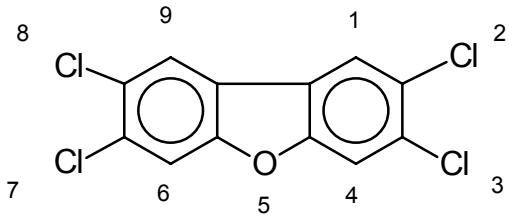
parameters could facilitate trace-back studies by providing a means to recognize patterns which may be attributed to specific sources. Little data is currently available for livestock animals. Studies into the mechanisms of dioxin absorption and deposition may also point to new techniques which can help to remediate body burdens after an exposure or prevent uptake in the first place. In preliminary studies, leanness-enhancing agents and chitosan supplements have shown some promise as methods to decrease dioxin body burdens. Other possible avenues to investigate are enzyme systems which may be induced to increase metabolism and excretion of dioxins, or microflora which could be exploited to degrade dioxins to less toxic compounds.

In the mean time, producing high quality lean meats and encouraging consumers to eat low-fat healthy diets will help to keep dioxin intake low and allow the U.S. food supply to remain the safest and most economical in the world.

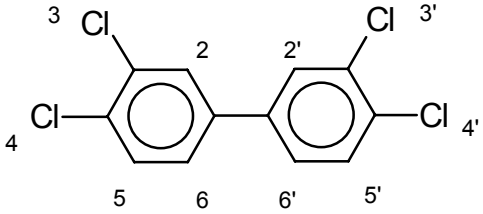
Figure 1. Structures and numbering of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran and 3,3',4,4'-tetrachlorobiphenyl.



2,3,7,8-Tetrachlorodibenzo-p-dioxin



2,3,7,8-Tetrachlorodibenzofuran



3,3',4,4'-Tetrachlorobiphenyl

PCDD/PCDF	1988 NATO TEFs	1998 WHO TEFs
2378-TCDD	1.0	1.0
12378-PeCDD	*0.5*	*1.0*
123478-HxCDD	0.1	0.1
123678-HxCDD	0.1	0.1
123789-HxCDD	0.1	0.1
1234678-HpCDD	0.01	0.01
OCDD	*0.001*	*0.0001*
2378-TCDF	0.1	0.1
12378-PeCDF	0.05	0.05
23478-PeCDF	0.5	0.5
123478-HxCDF	0.1	0.1
123678-HxCDF	0.1	0.1
123789-HxCDF	0.1	0.1
234678-HxCDF	0.1	0.1
1234678-HpCDF	0.01	0.01
1234789-HpCDF	0.01	0.01
OCDF	*0.001*	*0.0001*

PCB	1994 WHO TEFs	1998 WHO TEFs
Non-ortho PCBs		
33'44'-TeCB	*0.0005*	*0.0001*
344'5-TeCB	-	*0.0001*
33'44'5-PeCB	0.1	0.1
33'44'55'-HxCB	0..01	0.01
Mono-ortho PCBs		
233'44'-PeCB	0.0001	0.0001
2344'5-PeCB	0.0005	0.0005
23'44'5-PeCB	0.0001	0.0001
2'344'5-PeCB	0.0001	0.0001
233'44'5-HxCB	0.0005	0.0005
233'44'5'-HxCB	0.0005	0.0005
23'44'55'-HxCB	0.00001	0.00001
233'44'55'-HpCB	0.0001	0.0001
Di-ortho PCBs		
22'33'44'5-HpCB	*0.00001*	-
22'3'44'55'-HpCB	*0.00001*	-

Table 1. Comparison of the most common toxic equivalency factors for PCDDs, PCDFs, and PCBs used prior to 1998 and the most recently recommended. TEFs which differ are in italics.

	Fiedler (1997) MS, U.S.	Schecter (1997) across U.S.	Fürst (1990) Germany	Malisch (1998) Germany	Theelen (1993) Netherlands
beef	0.77 (0.67)	2.16 (1.90)	2.53 (1.69)	(0.71)	(1.75)
pork	0.75 (0.74)	2.61 (2.29)	0.64 (0.4)	(0.31)	(0.43)
chicken	0.78 (0.70)	3.90 (3.0)	1.89 (1.41)	(0.62)	(1.65)
dairy	0.96 (0.77)	2.69 (2.15)	1.69 (1.32)	0.80 (0.69)	(1.59)
eggs	0.29 (0.23)	2.11 (2.11)		2.38 (2.10)	(2.0)
farm-raised fish	27.1 (20.5)			(7.44)	
other fish	19.4 (15.6)	18.17 (15.88)	19.26 (14.87)	(37.54)	(19.23)
vegetable products		2.76 (2.21) vegan diet	>0.6 (<0.4) salad oil		(0.02) vegetable oils
non-detects	= LOQ/2	= LOD/2	= LOD	not reported	not reported

Table 2. Dioxin and furan TEQs on a lipid weight basis in food items (ppt). 1998 WHO TEFs were used to calculate TEQs and nondetects were treated as indicated in the last row. Values in parentheses are original data calculated using earlier TEFs.

	Fiedler (1997) MS, U.S.	Schecter (1997) across U.S.	Malisch (1998) Germany	Gilbert (1992) the U.K.
beef	0.22 (0.19)	0.28 (0.25)	0.14 (0.12) meats in general	0.79 (0.68) meats in general
pork	0.24 (0.23)	0.24 (0.21)		
chicken	0.07 (0.06)	0.21 (0.18)		0.37 (0.33)
milk	0.03 (0.02)	0.13 (0.10)	0.03 (0.02)	0.26 (0.21)
cheeses	0.33 (0.26)	0.33 (0.27)		0.18 (0.16)
butter	0.76 (0.61)	0.47 (0.52)	0.61 (0.53)	1.27 (1.07)
eggs	0.03 (0.02)	0.31 (0.31)	0.23 (0.20)	0.22 (0.19)
farm-raised fish	2.90 (2.19)		(0.33)	
other fish	0.35 (0.28)	0.55 (0.47)	(0.60)	0.57 (0.47)
vegetable products		0.08 (0.06) vegan diet	(0.015) vegetables	(0.05) vegetables
non-detects	= LOQ/2	= LOD/2	not reported	= LOD

Table 3. Dioxin and furan TEQs on a whole weight basis in food items (ppt). 1998 WHO TEFs were used to calculate TEQs and nondetects were treated as indicated in the last row. Values in parentheses are original data calculated using earlier TEFs.

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